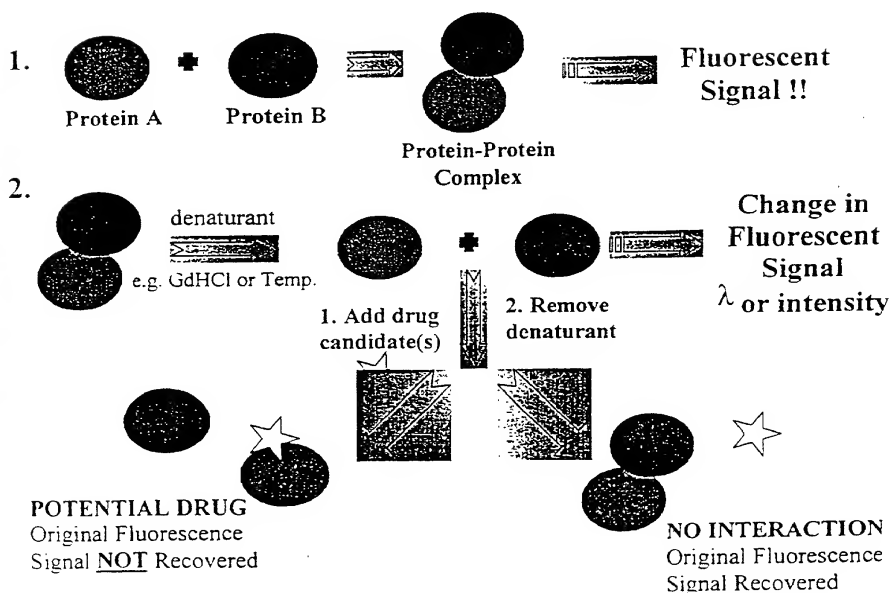


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FIGURE 1

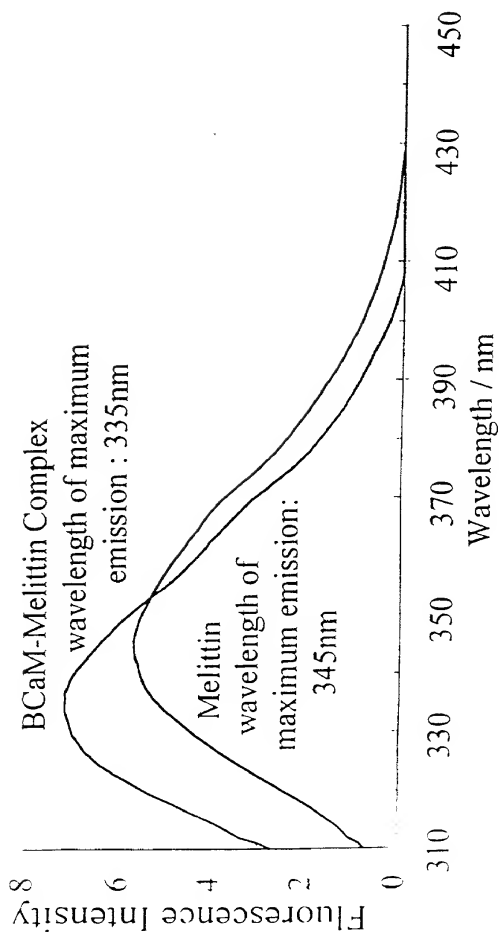
Proposed Assay



ENTRAP PROTEIN-PROTEIN COMPLEX IN A SOL-GEL
DERIVED GLASS

FIGURE 2

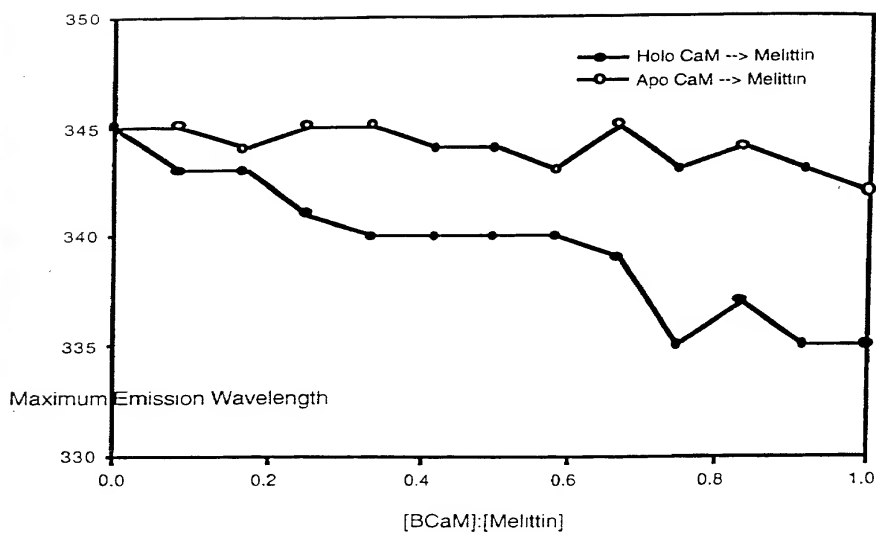
Fluorescence Signal from BCaM-Melittin System



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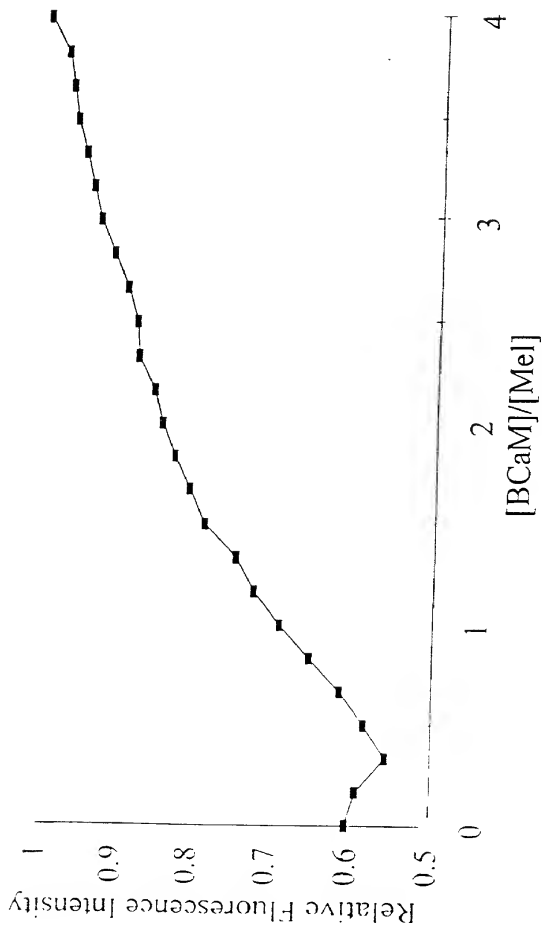
FIGURES 3



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FIGURE 4

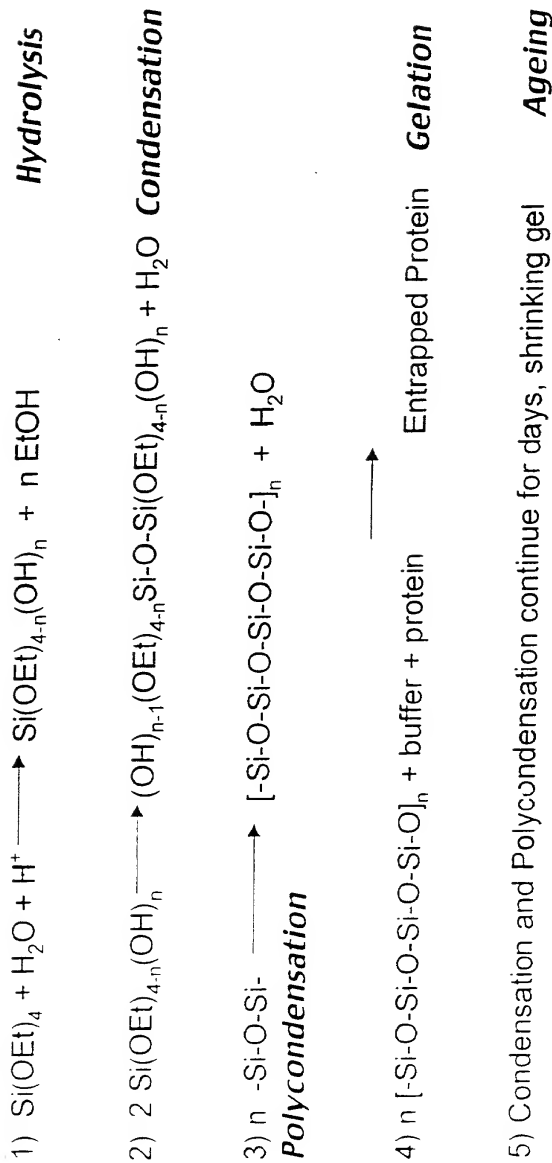


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FIGURE 5

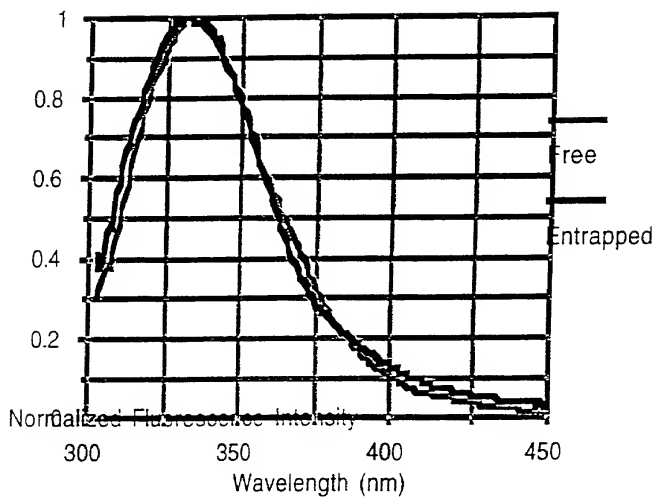
The Sol-Gel Process



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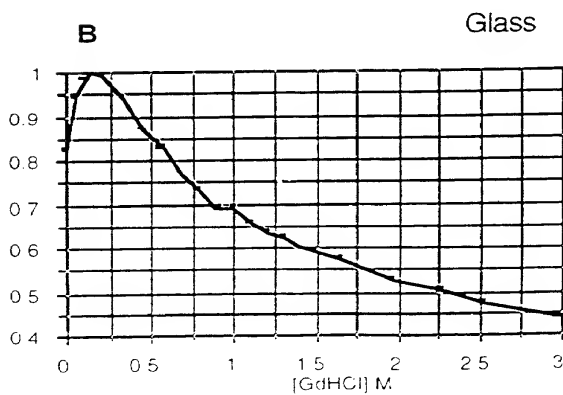
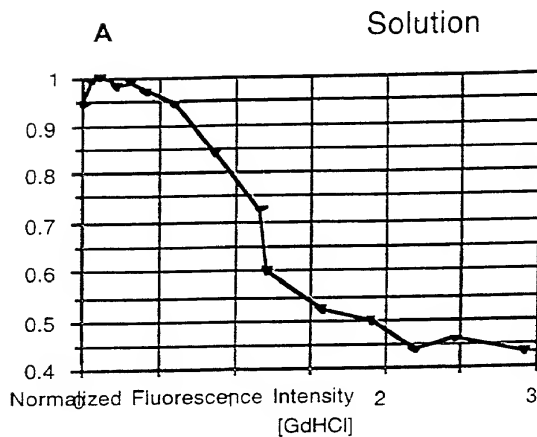
FIGURE 6



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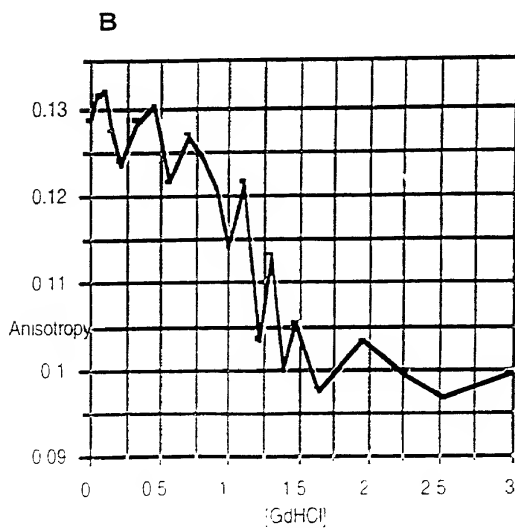
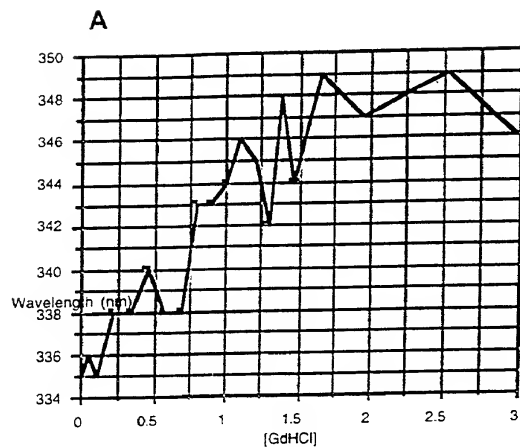
FIGURE 7A-B



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FIGURE 8A-B

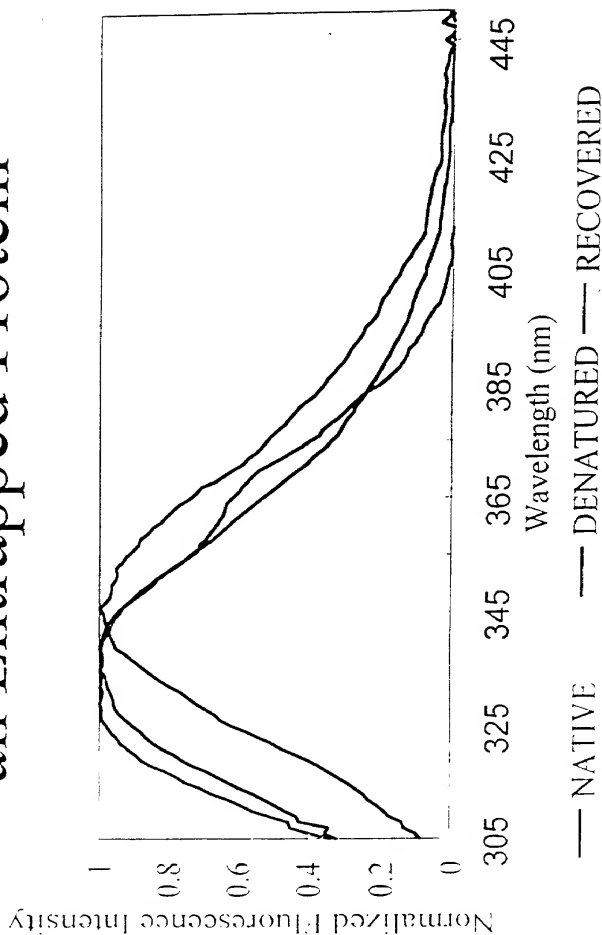


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FIGURE 9

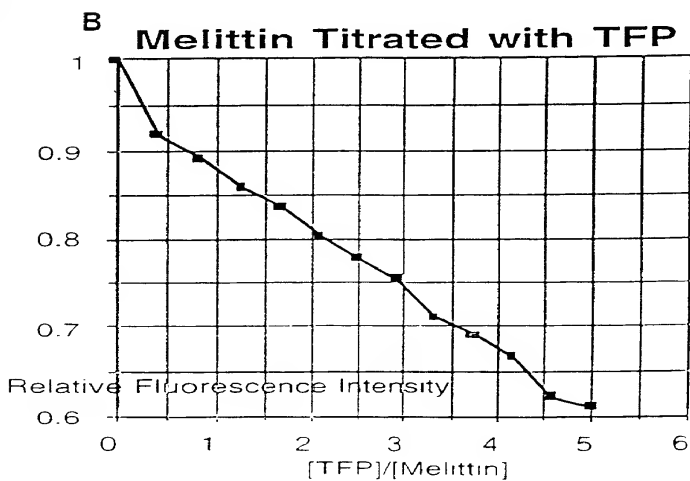
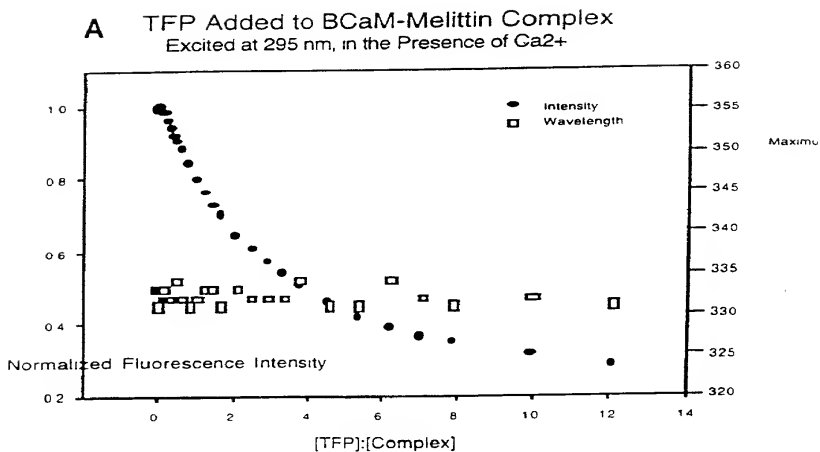
Reversibility of Denaturation for an Entrapped Protein



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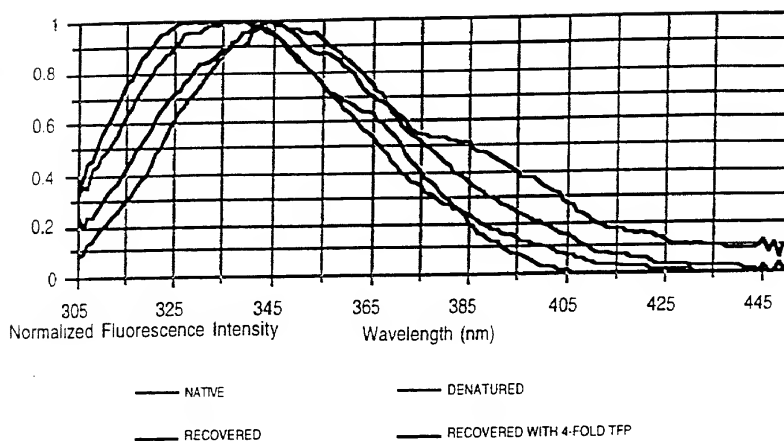
FIGURE 10A-B



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FIGURE 11



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FIGURE 12

Proof of Concept of the Assay

Solution	Anisotropy	Wavelength (nm)
Native	0.127	335
Denatured	0.129	334
Recovered	0.099	346
Denatured	0.136	331
Recovered with TFP	0.115	346
	0.121	346

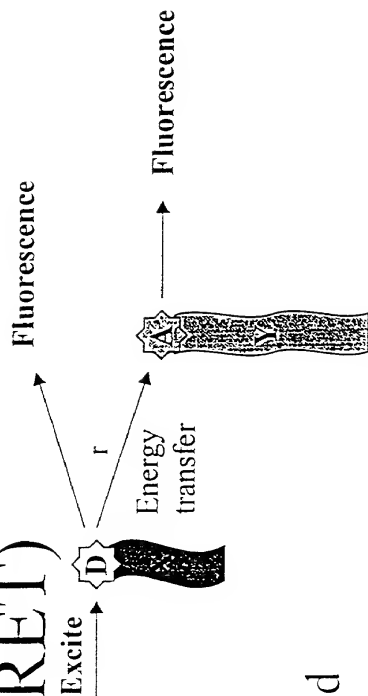
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FIGURE 13

Florescence Resonance Energy

Transfer (FRET)



- Donor excited
- Emits at λ_D that overlaps Acceptor absorbance
- Acceptor excited and emits at a longer λ_A
- Efficiency of energy transfer dependent on distance between donor and acceptor
- Förster distance (R_0) = 50% efficiency

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FIGURE 14

- Donor attached to Protein A, Acceptor attached to Protein B
- Efficiency of energy transfer (E) related to distance (r) by;
$$\frac{R_0^6}{R_0^6 + r^6}$$
- Use donor molecule which absorbs at longer λ to TFP \therefore avoid exciting TFP

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FIGURE 15

MelittinBCaM

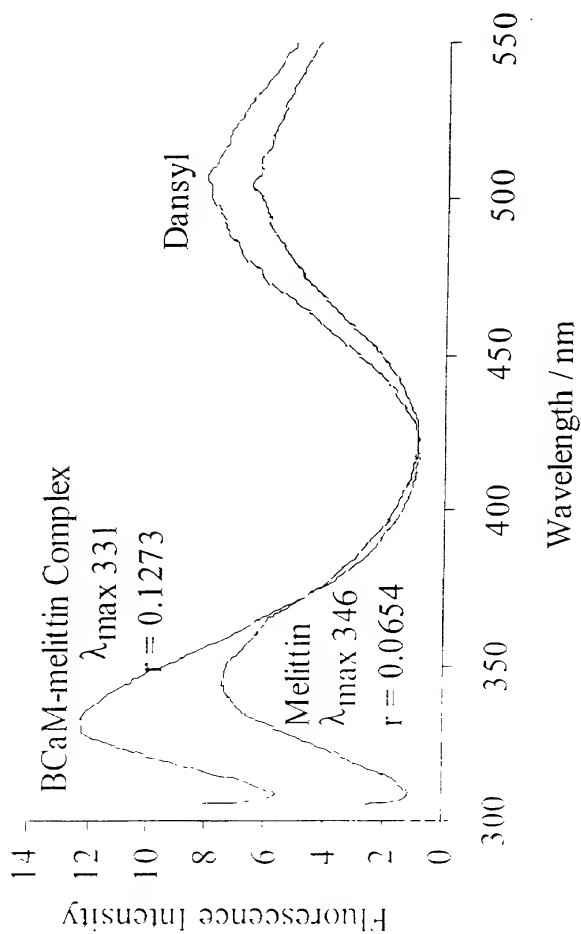
- Energy Donor
- Dansyl Chloride
- Absorbs @ 370nm
- Emits @ 500nm
- Energy Acceptor
- Fluorescein derivative
- Absorbs @ 494nm
- Emits @ 518nm

$$R_0 = 3.3 - 4.1 \text{ nm}$$

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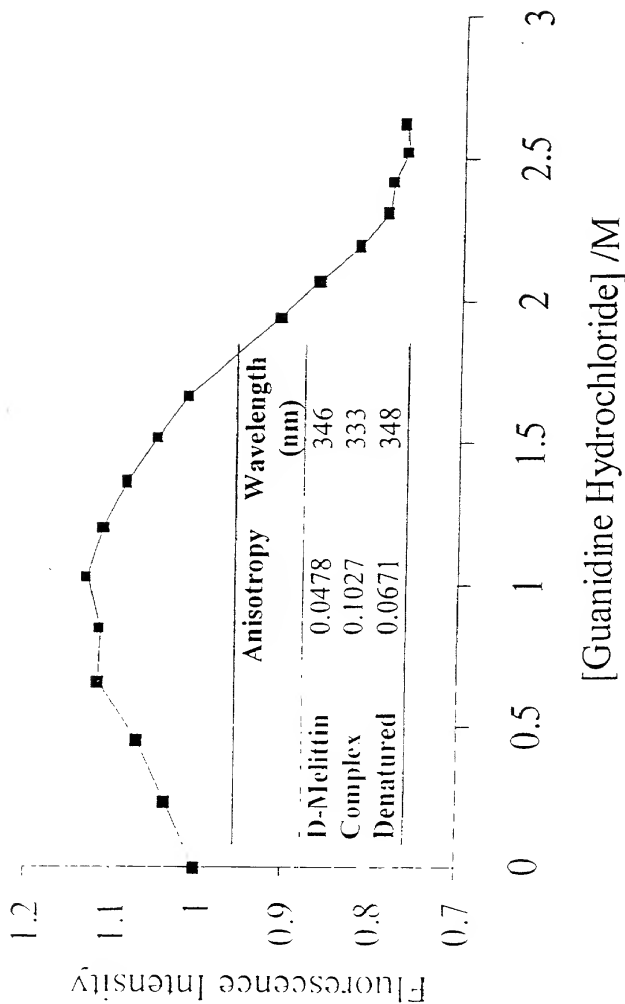
FIGURE 16



Dansyl doesn't interfere with PP interaction

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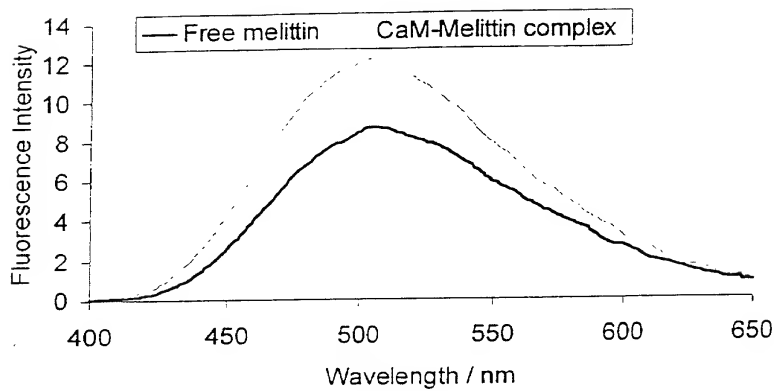
FIGURE 17



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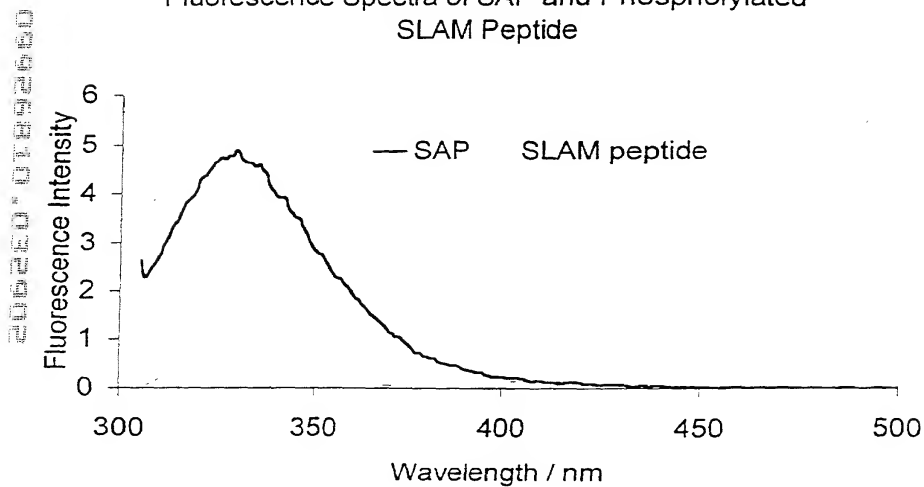
FIGURE 18



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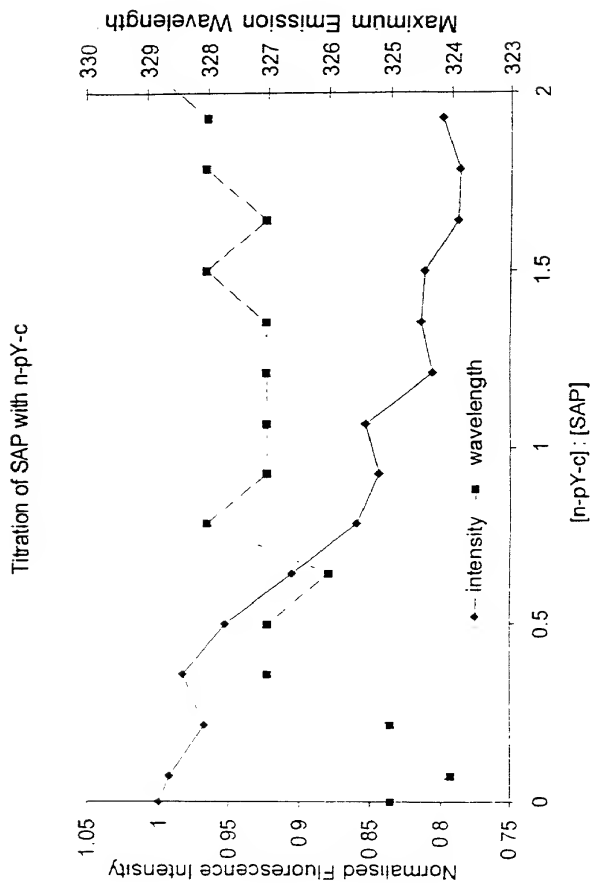
FIGURE 19

Fluorescence Spectra of SAP and Phosphorylated
SLAM Peptide

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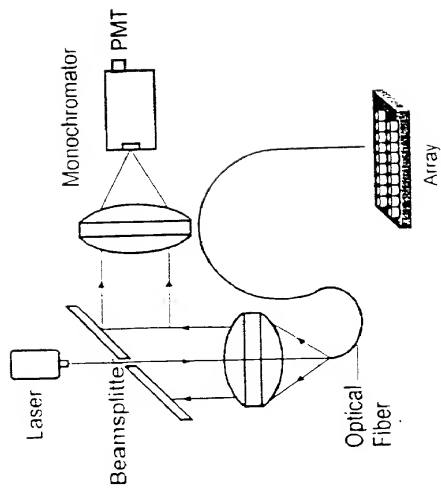
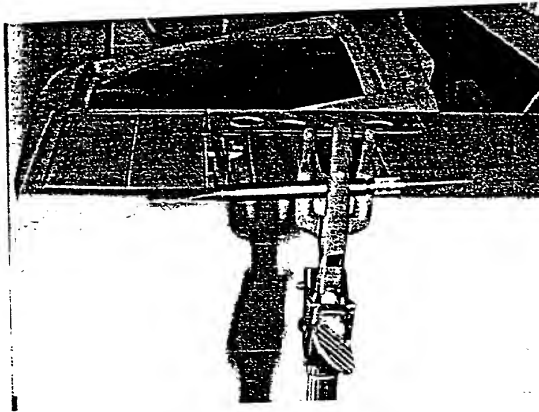
FIGURE 20



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FIGURE 21



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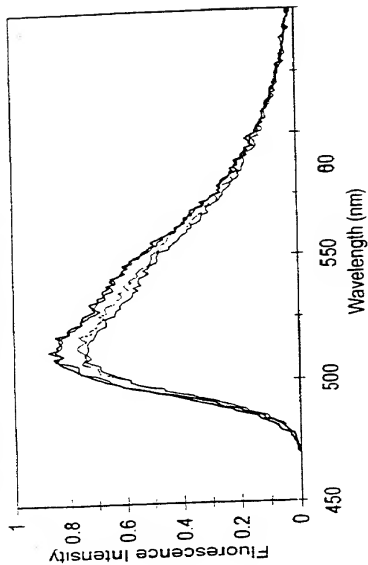
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FIGURE 22

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Intensity across Row 1



$2 \times 10^{-4} \text{ M}$
 $2 \times 10^{-5} \text{ M}$
 $3 \times 10^{-6} \text{ M}$
 $6 \times 10^{-8} \text{ M}$
 $8 \times 10^{-9} \text{ M}$
 $2 \times 10^{-9} \text{ M}$
 $2 \times 10^{-10} \text{ M}$
 $2 \times 10^{-11} \text{ M}$
 $2 \times 10^{-12} \text{ M}$

Intensity Down Column 3

